

IN THE CLAIMS:

Please cancel claims 14-185 without prejudice or disclaimer of subject matter.

REMARKS

This application is a division of Application No. 08/943,019, now allowed and is directed to restricted out claims 1-13 therein. The specification has been amended as in the parent application to correct the errors made of record therein.

In compliance with the duty of disclosure under 37 C.F.R. § 1.56 and in accordance with the practice under 37 C.F.R. §§ 1.97 and 1.98, the Examiner's attention is directed to the documents listed on the enclosed Form PTO-1449. Copies of the listed documents are found in the filewrapper of the parent application.

The concise explanation of relevance for the non-English documents is found in the specification where they are cited, or in an abstract, if attached.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should continue to be directed to our below listed address.

Respectfully submitted,



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191273

VERSION WITH MARKINGS TO SHOW CHANGES MADE TO SPECIFICATION

The paragraph at page 4, lines 6-14 has been amended as follows:

--In probe methods using radioisotopes, labels containing [radio active] radioactive atoms (radioisotopes) are used, and the detection sensitivity is satisfactory, and theoretically even one molecule (one copy) of the target nucleic acid can be detected.

Such probe methods using radioisotopes, however, require special facilities, and the operation is accompanied by dangers. Further, since radioisotopes are [instable] unstable, probe nucleic acids labelled with radioisotopes cannot be stably stored for long time periods.--

The paragraph beginning at page 4, line 15 and ending at page 5, line 2 has been amended with the following replacement paragraph.

--In contrast, probe methods using conventional chemical staining methods or enzymatic staining methods are more practical since they do not require special facilities, and the operation is relatively safe. Such probe methods using conventional staining methods are, however, markedly inferior in sensitivity to those using radioisotopes, and cannot sufficiently cope with detection of nucleic acids which can be obtained only in extremely small quantities, such as nucleic acids derived from organismal samples. Further, in many cases, probe nucleic acids bonded with labelling substances for

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such conventional staining methods are also [instable] unstable, and cannot be stored for long time periods.--

The paragraph beginning at page 5, line 25 and ending at page 6, line 3 has been amended with the following replacement paragraph.

93
--Furthermore, another object of the present invention is to provide a highly sensitive process for detecting a double-stranded nucleic acid in a sample with a simple procedure.--

94
The paragraph on page 6, lines 10-13 has been amended with the following replacement paragraph.

--According to an aspect of the present invention, there is provided a process for detecting a target single-stranded nucleic acid having a first base sequence [comprises] comprising the steps of:--

The paragraph beginning at page 6, line 25 and ending at page 7, line 3 has been amended with the following replacement paragraph.

95
--According to another aspect of the present invention, there is provided a process for quantifying a target single-stranded nucleic acid having a first base sequence [comprises] comprising the steps of:--

Please insert the following paragraph on page 16, after line 12 and before

line 13:

--As employed herein the term "attomole" means 10^{-18} mole. The symbol

"fM" is an abbreviation for femtomole or 10^{-15} mole.--

Please insert the following new paragraph on page 26, after line 25:

--Compound 2 in Table 1 may be made by the prior art synthesis described

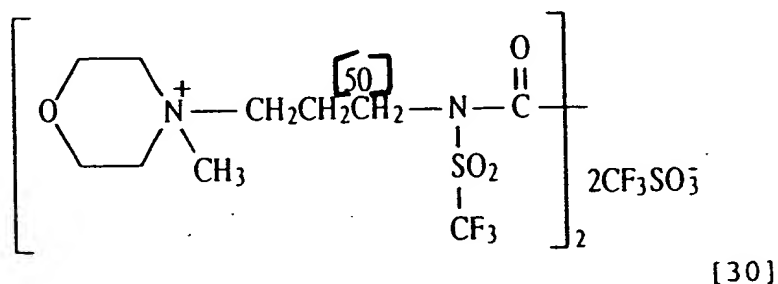
in column 18, line 4 to column 19, line 19 and column 20, lines 1-5 of U.S. Patent No.

5,624,798 issued April 29, 1997, the aforesaid disclosure of which is herewith incorporated

herein by reference.--

The structural compound [30] on page 51, lines 19-23 has been amended

with the following replacement structural compound [30] as follows.



The paragraph beginning at page 52, line 4 and ending at page 53, line 2 has

been amended with the following replacement paragraph.

9⁹

--Chemiluminescence can be detected in an appropriate medium capable of causing the chemiluminescent compound associated with a double-stranded nucleic acid to emit luminescence. An aqueous medium is preferred as a medium, since the medium can prevent the chemiluminescent compound in free state from emitting luminescence in the presence of a reagent causing the compound to emit luminescence. Examples of such a medium include water, aqueous buffered solutions, such as a phosphate buffered solution, Tris buffered solution, etc. The pH of such an aqueous medium preferably falls within a range in which the double-stranded nucleic acid and the chemiluminescent compound are stable. In the case of the compound represented by the general formula [1], the preferred pH range is from 5.0 to 8.0. Incidentally, although some of the compounds represented by the general formula [1] are capable of exhibiting chemiluminescence in a highly viscous organic solvent such as dimethyl phthalate in the presence of a luminescence-inducing reagent such as the combination of hydrogen peroxide and bisdinitrophenyl oxalate (Compound [59] 19) even in the absence of a double-stranded nucleic acid, such compounds do not exhibit chemiluminescence in the case where an aqueous medium is used instead of such an organic solvent.--

The paragraph at page 66, lines 12-18, has been amended with the following replacement paragraph.

9¹⁰

--Next, each 5 μ l of a TE buffered solution containing 1 picomole of a probe DNA, M13 Primer M4 SEQ ID No. 1 d(GTTTTCCTCCAGTCACGAC) (manufactured

a¹⁰
by Takara Shuzo Co., Ltd.), which was previously subjected to a heat-shock treatment at 90°C was added to each well. In this state, the microplate was covered, and hybridization was carried out at 60°C for 18 hours while shaking.--

The paragraph at page 73, lines 15-21, with the following replacement paragraph. A marked-up copy of this paragraph, showing the changes made, is attached.

a¹¹
--Next, each 5 µl of a TE buffered solution containing 1picomole of a probe DNA, M13 Primer M3 SEQ ID No. 2 d(GTAAAACGACGGCCAGT) (manufactured by Takara Shuzo Co., Ltd.), which was previously subjected to a heat-shock treatment at 90°C was added to each well. In this state, the microplate was covered, and hybridization was carried out at 60°C for 18 hours while shaking.--

The paragraph at page 79, line 23 has been amended with the following replacement paragraph.

a¹²
--SEQ.ID No. 3 5'-NH₂-ATGCTGGCCGTGACGCACAGCA-3'--

The paragraph beginning at page 83, line 21 and ending at page 84, line 1, has been amended with the following replacement paragraph.

a¹³
--The sequence of M13 primer M4 SEQ. ID No. 4 d(GTTTTCCCAGTCACGAC) is selected, and modified at the 5' end with an amino group based on the method of Example 11. The thus-synthesized probe DNA was immobilized to a microplate by covalent binding.

A new paragraph at page 1, line 5 has been added as follows:

-- (New) This application is a division of allowed Application No.

08/943,019, filed October 2, 1997.--

A new paragraph at page 16, after line 12 is as follows:

--(New) As employed herein the term "attomole" means 10^{-18} mole. The symbol "fM" is an abbreviation for femtomole or 10^{-15} mole.--

A new paragraph at page 26, after line 26 is as follows:

--(New) Compound 2 in Table 1 may be made by the prior art synthesis described in column 18, line 4 to column 19, line 19 and column 20, lines 1-5 of U.S. Patent No. 5,624,798 issued April 29, 1997, the aforesaid disclosure of which is herewith incorporated herein by reference.--

A new paragraph at page 83, after line 10 has been added as follows:

(New) This means that the weight of 1×10^{-21} moles of the target nucleic acid is 1 fg (femtogram).--

A Sequence Listing has been added after page 98 as follows:

(New)

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: TADASHI OKAMOTO
- (ii) TITLE OF INVENTION: PROCESS FOR DETECTING TARGET NUCLEIC ACID, PROCESS FOR QUANTIFYING THE SAME, AND PYRYLIUM COMPOUND FOR CHEMILUMINESCENCE ANALYSIS
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE:
FITZPATRICK, CELLA, HARPER & SCINTO
 - (B) STREET: 30 ROCKEFELLER PLAZA
 - (C) CITY: NEW YORK
 - (D) STATE: NEW YORK
 - (E) COUNTRY: UNITED STATE OF AMERICA
 - (F) ZIP: 10112-3801
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: DISKETTE: 3.50 INCH, 144 MB
STORAGE
 - (B) COMPUTER: IBM
 - (C) OPERATING SYSTEM: MS-DOS
 - (D) SOFTWARE: PATENTIN VER. 2.0
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/943,019
 - (B) FILING DATE: OCTOBER 2, 1997

(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTTTTCCCAG TCACGAC

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- (2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATGCTGGCCG TGACGCACAG CA

22

- (2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "SYNTHETIC RNA"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GTTTTCCCAG TCACGAC

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- (2) INFORMATION FOR SEQ ID NO: 4

- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTTTCCCAG TCACGAC

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